Monoterpenes in Plants- a mini review

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ABSTRACT

Monoterpenes are abundant chemicals in plants that are formed by a multitude of enzymes and present a challenge both in identification and understanding the synthetic enzymes involved in their formation and fate. They are especially abundant in fragrance-producing plants. The enzyme that produce them are numerous but several key enzymes have been identified and are discussed in this short review.

INTRODUCTION

Over 3,000 terpenoids are known [1] and they constitute the largest family of natural products, exceeding in number the alkaloids and phenylpropanoids combined [2]. Monoterpenes are 10-carbon members of the isoprenoid family of natural products [3]. Most members of this group are synthesized by the monoterpeno synthases (cycloas).

Monoterpeno synthases catalyze the conversion of the ubiquitous acyclic precursor, geranyl pyrophosphate (GPP) to the cyclic parents of the various monoterpeno skeletal types which are often subsequently transformed to a wide range of derivatives by various redox reactions [4].

Monoterpenes can be divided into three major subgroups: linear monoterpenes, monooxygen monoterpenes and bicyclic monoterpenes [5].

LINALOOL

Linalool is a naturally-occurring terpene alcohol chemical with many commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of spiciness). It is found in many flowers and spice plants including Michelia alba. It was found that 3.183% linalool can be identified through SPME technique and is one of the main compounds in Michelia alba volatiles [6].

STORAGE OF MONOTERPENES IN PLANT

The storage site of monoterpenes in plant are the oil glands, glandular hairs and trichomes cell of the leaf. The biological role of monoterpenes include herbivore defense. Animal ingesting the toxic monoterpenes experience pain and discomfort due to the action of the monoterpenes on cellular enzyme activity- chiefly on the mitochondrial enzymes. To have this effect the monoterpenes must be present at a substantial amount in the organs mention earlier. Consumption of animals lead to rupture of the glands and he monoterpenes will be released into the surrounding environment [7]. Monoterpeno emission rate in leaf depends upon its pool size in a particular plant and environmental variation such as temperature play an important parameter with a higher temperature generally resulted in a higher rate of emission. Due to this, plants need a large pool size but at the carbon expense for plant growth and reproduction [7].

Several factors that could affect the regulation of monoterpeno production include carbon balance, ratio of photosynthetic carbon assimilation and its utilization and plant wounding. In the latter, wounding triggers a large increases in monoterpeno synthesis in bark tissues. In addition, it is possible...
that other emission-related factors would be affected by the wounding treatment [8].

Broad leaf species that emit monoterpenes to the atmosphere harbor oil glands, glandular hairs and trichomes on the leaf surface. The basal emission rate is dependent upon the pool size of monoterpenes, physical resistance imposed by the tissue and the storage structure [9].

**MONOTERPENE BIOSYNTHESIS**

Terpene synthase converts the three universal intermediates of the isoprenoid pathway—geranylgeranyl diphosphate (GGPP), geranyl pyrophosphate (GPP) and farnesyl diphosphate (FPP) to diterpenes, monoterpenes and sesquiterpenes, through various mechanisms that include a common electrophilic reaction [10].

Some cyclases (pinane, bornane, fenchane, camphene and thujane families) were studied to formulate the general stereocchemical model of the biosynthesis of monoterpenes [4]. This multistep transformation of geranyl pyrophosphate, (GPP) is considered to involve the initial oxidation of the primary allylic ester to generate an ion pair that, by syn-migration of the pyrophosphate, provides the enzyme bound tertiary allylic isomer, either (3R)- or (3S)-inalyl pyrophosphate (LPP) depending on the stereochromy of the enzyme. Rotation about the newly formed C2-C3 single bond renders the bound intermediate topologically competent to cyclize, ionization and cyclization via the cisoid, anti-endo-conformer generates the corresponding monocyclic (4R)- or (4S)-α-terpinyl cation, respectively. The further course of the reaction from this universal intermediate may involve additional electrophilic cyclizations and rearrangements before termination of the catenionic reaction sequence [11].

Based on the studies of different monoterpen synthases and their biosynthesis pathways, this basic biosynthesis mechanism is proved to be responsible for the formation of about 30-40 monoterpenes from substrate geranyl pyrophosphate (GPP). Thus far, relevant chemical model studies and all investigation with cell-free cyclase preparations have fully supported this mechanistic scheme [7].

Linalyl diphosphate is an important intermediate in monoterpenene synthase catalysis. All studies to observe directly this product in the mandatory isomerization step have failed. One example of the research was the utilization of some noncatalytic substrate analogs. It is suggested that the highly tertiary allylic system is produced at the active site where ionization and cyclization occur. Preliminary attempts have been carried out using directed mutagenesis to dissect the cyclic isomerization step of the normally coupled reaction sequence by the monoterpenene cyclases have produced significant results [11].

Evaluation of monoterpenene synthases that carry out only this partial reaction are used to examine the isomerization step of the reaction. No linalyl diphosphate synthase has yet been described. However, two linalool synthases, capable of transforming a substrate geranyl pyrophosphate, (GPP) to the tertiary allylic alcohol as the sole product have been cloned. The 3S-linalool synthase of *Clarkia breweri* flower is involved in floral scent production in this species [8]. A detailed analysis of this linalool synthase gene suggests that it is a composite structure of recent origin, which more closely resembles a diterpene synthase than a monoterpenene synthase [8]. The noncatalytic substrate analogs 6,7-dihydrogeranyl diphosphate, (DHGPP) and racemic methanogeranyl diphosphate, (MGPP) have been used to dissect the cyclic isomerization step of the normally coupled reaction sequence but the inability to resolve chiral monoterpenene products and the limited product available from native monoterpenene synthases have plagued early researchers [12].

**ALTERNATIVE TERMINATION CHEMISTRIES OF MONOTERPENE SYNTHASE**

The first step of monoterpenene cyclization reactions include ionization and isomerization of the substrate geranyl diphosphate, (GPP). This is followed by the cyclization of bound linalyl diphosphate, (LDP) through a series of carbon intermediates. Ultimately, termination of the multistep cascade is carried out through processes such as deprotonation or nucleophile capture [13].

**SYMmetrical MONOTERPENOID**

1,8-cineole, 1,4-cineole and tricycene are some of the symmetrical monoterpenoids found. No absolute stereocchemical references can be made of the isomerization-cyclization cascade. Of these symmetrical types, 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane) is by far the most common, occurring in numerous essential oils. Given the relative stereocchemical elements of the suprafacial isomerization to the linalyl intermediate and the anti endo-cyclization of the latter, two possible stereocchemical routes to 1,8-cineole can be formulated. These two alternatives, mirror-image pathways can be distinguished by examining the fate of C1 of the geranyl substrate, through which asymmetry is introduced, and by evaluating the relative utilization of (3R)- and (3S)-inalyl pyrophosphate (and, potentially, (4R)- and (4S)-α-terpinyl) as alternate substrates [14].

![Monoterpene (menthol and carvone) biosynthesis pathway](image-url)
IMPORTANT AND FUNCTIONS OF MONOTERPENE

Monoterpenes are 10-carbon members belonging to the isoprenoid family of natural products. They are often responsible for the characteristic odors of plants and are widespread in the plant kingdom. Monoterpenes are used as flavorings, fragrances, and pharmaceuticals and this has stimulated works to increase their yield in plants [15,16]. As previously mentioned, monoterpenes act as semiochemicals in plant defense. In the Grand Fir (Abies grandis), at the site of injury, defensive oleoresin formation in conifers occurs through processes involving constitutive accumulation of resin (pitch) in specialized secretory structures followed by the biosynthesis of sesquiterpenes (turpenite), monoterpenes and diterpene resin acids in nonspecialized cells. Following the evaporation of the monoterpenone solvent, the mixture is hardened to form a mechanical barrier that seal the wound or the infected site [10].

In marine red alga Octodes secundiramea, the acyclic monoterpenone myrcene is the progenitor of the unusual cytotoxic halogenated monoterpenes that function as feeding deterrents to herbivore. These compounds often possess unusual biological activities consistent with their roles in the chemical defenses of the soft bodied, sessile inhabitants of this highly competitive environment, and several have already found important biomedical applications [17]. The response of plants towards various stress factors is also mediated by secondary metabolites that include monoterpenes. The role of terpenes in forest decline phenomena has also been studied [18]. Monoterpenes of commercial value include their use as industrial raw materials, essential oil for perfumery and flavoring. For example, lemon essential oil is widely used as flavoring agent in bakery, pharmaceutical applications and as fragrance in perfumery [19].

The famous turpenite (consisting of monoterpenone) is source of industrial chemical intermediate and a high-grade commercial solvent [20,21]. Other examples include is Perilla frutescens Britton (Labiatae), which is used as a food, Chinese crude drug, natural pigment, and spice in Asian countries [22]. The essential oil from Eucalyptus, chiefly 1,8-cineole, is used for medicinal and pharmaceutical purposes [23]. Previous research also revealed that the benzyl ether derivatives of 1,8-cineole could be used as agrochemicals [24]. The caraway fruits contain the monoterpenone (+)-carvone can be used as an effective sprouting inhibitor of potatoes [15]. Traditionally, monoterpenone rich essential oils have been used for treatment of disease and maladies. However, the development of new and more effective drugs quickly replaced the monoterpenone containing mixture [24].

The natural emission of turpenite monoterpenones, such as a-pinene, from the forest trees is a major source of tropospheric organic compounds, upon oxidation have significant consequences for the ozone balance. For this reason, the biogenesis of turpenite monoterpenones and their genetic origins have received considerable recent attention [20].

STUDIES ON MONOTERPENE SYNTHASES

Several studies on the monoterpenone biosynthesis pathways from various plant sources were conducted by researchers around the world [7,8,25]. The biosynthesis of the monoterpenones, limonene and carvone in the fruit of caraway (Carum carvi L.) proceeds from geranyl pyrophosphate, (GPP) via a three-step pathways. The pathway of (+)-limonene and (+)-carvone biosynthesis in caraway has been assumed to be analogous to the biosynthesis of (+)-limonene and (+)-carvone in spearmint. In this process, GPP is the ubiquitous precursor of the monoterpenone [15]. Another molecular biological study on limonene synthase, that catalyzes the cyclization of geranyl pyrophosphate to yield the olean 4(S)-limonene, an intermediate in the biosynthesis of a monoterpenoid, perillaaldehyde, in Perilla frutescens has also been carried out [22].

A study has been carried out to observe the scent in the flowers of Clarkia breweri. The strong, sweet fragrance consists of at least 12 volatiles that fall into two groups, derivatives of the benzene pathway and monoterpenoids. Linalool forms a major component of the scent of C. breweri flowers. It is an acyclic monoterpenone alcohol common to the floral scents of many plant species. In addition to linalool, C. breweri flowers emit two linalool oxides that are precursors of linalool. The enzyme-S-linalool synthase (LIS) activity in flower parts was previously observed catalyzes the cation-dependent and stereoselective conversion of GPP to S-linalool [8,26].

(−)-Sabine is the major monoterpenone produced by the liverwort Conophyllum concinnum. A cell-free crude extract of cultured plants in vitro was found to catalyze the cyclization of GPP to sabine [27]. Another liverwort- Riccioecarpus natans contain 4S-(−)-limonene as a major monoterpenone and a cell-free extract from this nonvascular plant cultured in vitro catalyzes the cyclization of GPP to limonene with limonene synthase [28].

In the study of the biosynthesis of marine natural products, isolation and characterization of mycene synthase, a monoterpenone synthase, from cultured tissues of the marine red alga Octodes secundiramea was conducted [17]. The conversion of the monoterpenone precursor geranyl pyrophosphate to mycene represents the first in vitro demonstration of a monoterpenone synthase from any marine source and provides a basis with which to further explore the biosynthesis of halogenated monoterpenones [17].

In the study of characterization and functional expression of monoterpenone synthases from Arabidopsis thaliana, functional expression of several Arabidopsis species synthase like sequences in yielded an active monoterpenone synthase enzyme in Escherichia coli, that catalyze the conversion of GPP into the acyclic monoterpenones (E)-β-ocimene, β-myrcene and small amounts of cyclic monoterpenones [29]. Similar studies have been carried out in the lodgepole pine (Pinus contorta) to further define specific structural and mechanistic differences among monoterpenone synthases from divergent plant sources [30].

MONOTERPENE SYNTHASE FROM DIVERGENT PLANT SOURCES (GYMNOSPERMS AND ANGIOSPERMS)

Different enzyme properties are expected for monoterpenone synthases from divergent plant sources. But in general, they usually catalyze the stereospecific isomerization of GPP to 3R- or 3S-linalyl pyrophosphate. This is followed by cyclization reaction products related to the chirality of the linalyl intermediate. However, amino acid-specific chemical-modifying reagents have been demonstrated to act differently to this enzyme from gymnosperms and angiosperms [31].

An arginyl residue at the active site of monoterpenone synthase from gymnosperms has been found. A GGP synthase from Taxus canadensis was cloned, expressed and characterized and was found to harbor similar requirement for the amino acid at the active site. Since gymnosperms produce terpenes, they must
have substantial prenyltransferase activities involved in the secondary metabolism of terpenoids [31].

Monoterpenes synthase (Prenyltransferase) from numerous angiosperm species such as 4S-limonene synthase from peppermint and the pinene synthases from sage have been purified and characterized [31]. In general, monoterpenes synthase from angiosperms requires histidine and cysteine residues at the active site. In addition they have at least some catalytically important arginyl residues at a site not protected by substrate [30].

LOCATIONS OF MONOTERPENE SYNTHASES IN PLANT

The plastids in plants are the site of geranyl pyrophosphate (GPP) synthase and monoterpenes synthase. S-linalool, is abundant in the stigma of freshly opened flowers of Clarkia breweri [8]. Similarly, lipophilic metabolites such as monoterpenes from the liverwort Conocephalum conicum are deposited in specialized intracellular oil bodies [28]. Compartmentation of these enzymes in specialized organelles offer an explanation for the lack of production of monoterpenes in plant cell tissue cultures. Constitutively expressed olerocin is synthesized in the epithelial cells of specialized secretory structures in some plant species such as the comifer. Induced oleocin appears to originate from nonsecreted cells. Oleocin is composed of terpentine, largely monoterpenes with some sesquiterpene olefins [16].

In spearmint (Mentha spicata), peppermint (Mentha piperita), and other essential oil plants from the Lamiaceae, monoterpenes are produced and accumulated in specialized glandular trichomes. There are two types of nonphotosynthetic glandular trichomes in mint. The first is a small capitate type that have a limited capacity to store secreted material, and the second is the peltate type that contains a stalk cell, a basal cell, and eight secretory cells that are arranged in a disc. The peltate type develops a large oil-storage space in the glandular trichome and is responsible for the production of the bulk of the monoterpenes essential oil [21]. The secretory cells of isolated peppermint peltate glandular trichomes are responsible for the secretion of monoterpenes into the oil-storage space and also served as the actual site for the biosynthesis of monoterpenes. Oil gland sepalas are composed of nonpigmented plastid with few internal membranes and have been implicated in monoterpenes biosynthesis in nearly 50 plant species [13].

Leucoplast and other plastids, when isolated, are capable of monoterpenes biosynthesis when supplied with exogenous precursors. The glands of the fruit flavedo from Lemon (Citrus limon) possesses a high content and large variety of monoterpenes. This layer contains the epidermis that covers the exocarp. The latter is consisted of irregular parenchymatous cells, and are completely enclosed glands or oil sacs. In maturing fruits, just beneath the green layer is a thick spongy white mass of tissue of the albedo layer (mesocarp) that are rich in pectins [19].

GERANYL PYROPHOSPHATE SYNTHASE

Geranyl pyrophosphate synthase (GPPS) catalyzes the condensation of dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) to generate geranyl pyrophosphate (GPP), the essential precursor of monoterpenes biosynthesis. This enzyme is similar to farnesyl diphosphate synthase (FPPS) which condenses two molecules of IPP with DMAPP to form the C15 precursor of the sesquiterpenes and triterpenes and to geranylglycerol pyrophosphate synthase (GGPPS) which condenses three molecules of IPP with DMAPP to form the C20 precursor of diterpenes and tetraterpenes. These enzymes, referred to collectively as short-chain prenyltransferases, function at the branch points of isoprenoid metabolism. These enzymes are considered to play a regulatory role controlling the flux distribution of IPP into the various terpenoid families. In addition, all of the short-chain prenyltransferases share sufficient primary sequence identity to suggest that they are evolutionarily related [21]. Their catalytic mechanism involves the divalent metal ion-dependent ionization of the allylic co-substrate and 1'-4 addition to IPP. The product are higher isoprenologues.

Figure 3: Chemical structure of geranyl pyrophosphate (GPP).

A study of geranyl pyrophosphate synthase from Abies grandis, including the cDNA isolation, functional expression, and characterization was conducted. The pairwise comparison between the grand fir GPP synthase and GGPP synthase suggests that relatively few residues determine the chain length specificity of these prenyltransferases and encourages a direct mutagenesis approach to explore the structure-function differences. Directed mutagenesis of an FPP synthase to produce GPP and GGPP, and mutagenesis alteration of a GGPP synthase to produced FPP, have been achieved. However, the mutagenesis of a GGPP synthase to produce GPP has not yet been demonstrated [21]. GPP synthase from the principal precursors of the oleocin monoterpenes, were isolated, partially purified and characterized from the stems of 2-year-old grand fir saplings [31]. This shows the variety of enzymatic synthesis route in plants that lead to the numerous monoterpenes found.

REFERENCES


